

On page 42, line 8, please add --mM-- before the first word (Tris).

On page 42, line 11, please delete "50 Tris" and add --50 mM Tris--.

On page 42, line 12, please delete "5 EDTA" and add --5 mM EDTA--.

On page 42, line 15, please delete "50 Tris" and add --50 mM Tris--.

On page 42, line 16, please delete "50 EDTA" and add --50 mM EDTA--.

On page 42, Example 11, line 5, please delete "50 Tris-C1" and add --50 mM Tris-C1--.

On page 42, Example 11, line 5, please delete "5 EDTA" and add--5 mM EDTA--.

On page 42, Example 11, line 6, please add --mM-- before the first word (dithiothreitol).

REMARKS

New claims 29-39 are pending in the application. No new matter is added by these amendments. The new claims find full support throughout the specification and in the original claims.

Based on the following remarks, Applicants respectfully request that the Examiner reconsider all outstanding objections and rejections and withdraw each. The specification has been amended to set forth the status of Applicants' related applications and to correct informalities. No new matter is added by these amendments.

Introduction

Applicants have discovered that highly purified saponin adjuvants may be derived from Quillaja saponaria. The newly presented claims more clearly define Applicants' invention. Quillaja saponaria is the saponin source used in all of the patent application examples. In contrast to available mixtures, Applicants' preparations represent pure saponins adjuvants, without the toxicity associated with crude saponin adjuvant extract. As discussed in detail in the Rule 132 Declaration by Dr. Charlotte Kensil, one of the inventors of the claimed invention, until these purified saponins adjuvants were prepared and tested by Applicants, it would not have been expected that single components of the Quillaja saponaria extract would be effective adjuvants.

Applicants' invention, therefore, comprises novel pure saponin adjuvants with properties and characteristics that differ from those of the saponin mixture from which the compounds are isolated and purified.

New claims 29- 35 are directed to substantially pure saponin adjuvants. Claim 36 is directed to a method of enhancing an immune response which uses the saponin adjuvants. Claims 37-39 are directed to pharmaceutical preparations using the substantially pure saponin adjuvant. The method claims have been cancelled, without reservation, for filing in a continuation application.

Objection to the Disclosure and Claims

The disclosure was objected to because of a spelling error and missing units designations. These informalities have been corrected. The objections to the claims are also believed to be mooted in the newly submitted claims.

Rejections Under 35 U.S.C. § 112, Second Paragraph

The Examiner rejected certain claims under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the Applicants regard as the invention. These rejections are believed to be addressed by the newly submitted claims, thereby rendering this rejection obviated. Therefore, the Examiner is respectfully requested to reconsider this rejection and to withdraw it.

Rejections Under 35 U.S.C. § 103

Claims 4-28 were rejected under 35 U.S.C. § 103 as being unpatentable over Higuchi *et al.* (Phytochemistry 26:229-235) or Scott *et al.* (Int. Archs. Allergy Appl. Immun. 77:409-412) in combination with Combier *et al.*, Toyo Jozo, Kishimoto Sangyo, Nagasawa *et al.*, or Zhou *et al.* The Examiner stated that Higuchi *et al.* and Scott *et al.* teach the mixture of saponin adjuvants in the bark extract of Quillaja, and that it is well known that the extract contains a mixture of saponins. Applicants respectfully traverse this rejection. This rejection will be responded to in view of the newly submitted claims.

Applicants respectfully submit that neither Higuchi *et al.* nor Scott *et al.* teach the existence of "impurities" in Quillaja bark extract, nor do they teach that the extract contains toxic substances, as suggested by the Examiner. Higuchi *et al.* disclose the structure of quillajasaponin components in "an attempt to isolate the physiologically active triterpenoid compounds." (Higuchi *et al.*, p. 229) This reference does not teach the isolation of compounds comprising purer and less toxic saponin preparations.

Scott *et al.* describe the adjuvant activity of the mixture of triterpene glycosides from Quillaja bark. The reference does not suggest separation of the biologically active saponins from impurities or toxic substances in the mixture.

Further, these references are discussed in more detail in the attached Declaration of Dr. Kensil.

Applicants respectfully suggest that it would not be obvious to purify the saponin adjuvant mixtures of Scott *et al.* or Higuchi *et al.* Neither of these references suggests doing so, nor do they suggest that the adjuvant activity would be in any way enhanced or improved using a purer form of the saponin extract.

None of the secondary references cited by the Examiner suggests application of the purification steps for purifying saponin adjuvants derived from Quillaja. As discussed above, Combier *et al.* do not perform a further separation of the saponin extract from chrysanthemum, so this reference does not teach the purification of "various saponins", as suggested by the Examiner.

Because neither of the primary references discloses a lack of effectiveness or the existence of toxicity, neither do they teach a need to obtain saponins adjuvants free from toxins or impurities. One skilled in the art is not taught to apply the separation methods of any of the secondary references to the saponin mixtures disclosed by the primary references.

Rejections Under 35 U.S.C. § 102(b)

Claims 16-20 and 27 were rejected under 35 U.S.C. § 102(b) as being anticipated by Dalsgaard (Archiv. für die gesamte Virusforschung 44:243-254). According to the Examiner, Dalsgaard discloses a purified saponin adjuvant which is less toxic than crude Quillaja extract, and use of this adjuvant to enhance the immune response to an antigen. Applicants respectfully traverse this rejection. This rejection will be responded to in view of the newly submitted claims.

Applicants respectfully submit that the Dalsgaard reference does not anticipate the claims of the present invention. On p. 249 of the Dalsgaard reference it is stated, "no identification of the composition of Quil A has yet been performed, but it is believed that it is a saponin..." (emphasis added). At page 252 it is stated that Quil A is isolated in "virtually pure state."

It is respectfully submitted that the Quil A material disclosed by Dalsgaard is in fact a mixture of components as pointed out in Dr. Kensil's Declaration. When used as an adjuvant this mixture is toxic in mice and cats (specification, page 6). The fact that Quil A is a mixture extracted from natural plant material strongly suggests that the composition will vary among different preparations. The relative concentration of the components is liable to fluctuate, and the toxicity and effectiveness will not be identical in different preparations.

Unlike the Quil A preparation disclosed by Dalsgaard, the saponin adjuvants of the present invention are chemically pure as indicated by carbohydrate composition, UV absorption, retention time on reverse phase-HPLC, and other parameters.

For the above reasons, it is respectfully submitted that the highly purified saponins of the present invention are not anticipated by the saponin preparation disclosed by Dalsgaard.

Rejections Under 35 U.S.C. § 103

Claims 18-28 were rejected under 35 U.S.C. § 103 as being unpatentable over Dalsgaard, Nunberg, Freidrich-Loeffler or Buchnev. Applicants respectfully traverse this rejection. Again, this rejection will be responded to in view of the newly submitted claims.

Applicants have, for the first time, isolated chemically pure preparations of the saponin adjuvants extracted from Quillaja bark. Until the Applicants showed that these pure preparations possessed adjuvant activity, it was not obvious that removal of the majority of other components from the crude extract would not also remove the biological activity.

As indicated by Dalsgaard, a crude extract of Quillaja bark, Quil A, was thought to be pure and was only "thought" to be a saponin. In fact, Applicants have shown that Quil A causes severe damage to the livers of mice, and induces a pyrogenic response in kittens that persists for several hours. (Specification, page 48).

There is no teaching in Dalsgaard that it would be advantageous to attempt further separation and purification of the Quil A preparation. Thus the saponin adjuvants of the present invention, namely pure saponin adjuvants with consistent chemical properties, are not rendered obvious by the Dalsgaard reference.

The Nunberg reference simply points out what is known in the art, namely the combination of a protein or immunogen with an "appropriate adjuvant" (column 3, line 4). At column 17, lines 1-6, examples of adjuvants are given, but neither saponins nor carbohydrate-containing adjuvants are suggested.

Freidrich-Loeffler discloses a concentrated vaccine against foot and mouth disease; one component of the vaccine mixture is "saponin." Again, this is merely a suggestion to use an adjuvant known in the art. This reference does not render obvious the novel chemically pure saponin compounds disclosed by the Applicants.

Because it was not obvious that the purified saponins of the invention would possess adjuvant activity, combination of the pure products with antigen to enhance the immune response would not be expected to be successful. Therefore, Applicants respectfully submit that, although Nunberg, Freidrich-Loeffler and Buchnev may suggest the general use of adjuvants to enhance the immune response, there is no suggestion in these references to use pure preparations of saponins as adjuvants. Until Applicants isolated chemically pure Quillaja saponins and demonstrated that they could function as saponin adjuvants, it was not possible to combine these compounds with antigens.

CONCLUSION

All of the stated grounds of rejection have been properly traversed, accommodated or rendered moot. In view of the newly submitted claims, the remarks made above, and the statements made in Dr. Kensil's Declaration, Applicants request that the Examiner reconsider all presently outstanding rejections, and that she withdraw them. The Examiner is invited to telephone the undersigned representative if she believes that an interview might be useful for any reason.

Respectfully submitted,

SAIDMAN, STERNE, KESSLER & GOLDSTEIN

Hollie L. Baker
Hollie L. Baker
Attorney for Applicant
Registration No. 31,321

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1225 Connecticut Avenue
Washington, D.C. 20036
(202) 833-7533

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